ier mean ion mass at high latitudes. At low latitudes the topside measurements have shown the detailed latitudinal structure of the equatorial anomaly, demonstrating control by the geomagnetic field.

A variety of electron-density irregularities have been studied. Most are greatly elongated along the magnetic field, and produce echoes either by lateral scattering, if they are thin, or by longitudinal ducting, if they are thick. Some of the thick irregularities are continuous between the hemispheres and support conjugate echo propagation.

The topside sounders have revealed the complex structure of the ionosphere near the auroral zone and at higher latitudes. At night an east-west trough of greatly reduced electron density occurs equatorward of the auroral zone. At the auroral zone itself the electron density is high and quite variable, both in space and time. The electron density at the polar cap within the auroral zone is often uniform and smooth. Ionospheric irregularities are common in the area of the trough and the auroral zone.

Among other satellites, the topside sounders have been used in various plasma studies involving the excitation and propagation of waves. These studies suggest that the ionosphere is an appropriate region for future plasma physics investigations, especially with rocket and satellite payloads designed specifically for that purpose.

References

1. J. A. Ratcliffe, *Physics of the Upper Atmosphere* (Academic Press, New York, 1960); K. Davies, *Natl. Bur. Std. U.S. Monograph 80*, "Ionospheric Radio Propagation" (1965); C. O. Hines, I. Paghis, T. R. Hartz, J. A. Fejer, *Physics of the Earth's Upper Atmosphere* (Prentice-Hall, Englewood Cliffs, N.J., 1965); R. E. Bourdeau, *Science* **148**, 585 (1965).
2. J. A. Ratcliffe, *The Magnetoionic Theory* (University Press, Cambridge, England, 1962); K. G. Budden, *Radio Waves in the Ionosphere* (University Press, Cambridge, England, 1961).
3. G. L. Nelms, *Can. J. Phys.* **41**, 202 (1963); P. R. Arendt, *Nature* **208**, 443 (1965); A. C. Aikin and S. J. Bauer, in *Introduction to Space Science*, W. N. Hess, Ed. (Gordon and Breach, New York, 1965), p. 133.
4. W. B. Hanson, in *Space Research*, W. Priester, Ed. (North-Holland, Amsterdam, 1963), vol. 3, p. 282.
5. J. E. Jackson, in *Electron Density Distribution in the Ionosphere and Exosphere*, E. Thrane, Ed. (North-Holland, Amsterdam, 1964), p. 325; J. H. Chapman, in *Progress in Radio Science*, G. M. Brown, Ed. (Elsevier, Amsterdam, 1965), vol. 3, p. 264.
6. S. C. Gladden, *Natl. Bur. Std. U.S. Tech. Note 28* (1959).
7. R. W. Knecht, T. E. VanZandt, S. Russell, *J. Geophys. Res.* **66**, 3078 (1961); R. W. Knecht and S. Russell, *ibid.* **67**, 1178 (1962); W. Calvert, T. E. VanZandt, R. W. Knecht, G. B. Goe, in *Proceedings of the International Conference on the Ionosphere*, A. C. Stickland, Ed. (Inst. of Physics and the Physical Soc., London, 1963), p. 324.
8. E. S. Warren, *Can. J. Phys.* **40**, 1692 (1962); J. H. Chapman, *J. Spacecraft Rockets* **1**, 684 (1964).
9. G. L. Nelms, R. E. Barrington, J. S. Belrose, T. R. Hartz, I. B. McDiarmid, L. H. Brace, *Can. J. Phys.*, in press.
10. W. Calvert, R. W. Knecht, T. E. VanZandt, *Science* **146**, 391 (1964).
11. K. L. Bowles, *ibid.* **139**, 389 (1963).
12. The three books in reference (*1*) describe a wide variety of direct measurements and discuss their results, as does *Introduction to Space Science*, W. N. Hess, Ed. (Gordon and Breach, New York, 1965).
13. The Defence Research Telecommunications Establishment (Shirley Bay, Ottawa, Ontario, Canada) currently publishes Alouette I processed data in two forms: *ALOSYN* contains directly scaled local and peak quantities, *N(h)* contains complete vertical electron-density profiles. The NASA Ames Research Center (Palo Alto, Calif.) will be publishing vertical electron-density profiles.
14. R. Cohen and T. E. VanZandt, in preparation.
15. R. W. Knecht and T. E. VanZandt, *Nature* **197**, 641 (1963); W. Calvert, H. Rishbeth, T. E. VanZandt, *IG Bull. 83*, reprinted in *Trans. Am. Geophys. Union* **45**, 398 (1964); J. O. Thomas, M. J. Rycroft, L. Colin, K. L. Chan, in *Electron Density Profiles in the Ionosphere and Exosphere*, J. Frihagen, Ed. (North-Holland, Amsterdam, 1966), p. 322.
16. G. E. K. Lockwood and G. L. Nelms, *J. Atmospheric Terrest. Phys.* **26**, 569 (1964); J. W. King, P. A. Smith, D. Eccles, G. F. Fooks, H. Helm, *Proc. Roy. Soc. London Ser. A* **281**, 464 (1964).
17. T. E. VanZandt, R. B. Norton, H. Rishbeth, private communication; R. J. Moffett and W. B. Hanson, *Nature* **206**, 705 (1965); E. N. Bramley and M. Peart, *J. Atmospheric Terrest. Phys.* **27**, 1201 (1965).
18. L. E. Petrie, *Can. J. Phys.* **41**, 194 (1963); G. E. K. Lockwood and L. E. Petrie, *Planetary Space Sci.* **11**, 327 (1963); W. Calvert and C. W. Schmid, *J. Geophys. Res.* **69**, 1839 (1964).
19. D. B. Muldrew, *J. Geophys. Res.* **68**, 5355 (1963).
20. B. T. Loftus, T. E. VanZandt, W. Calvert, *Ann. Geophys.*, in press.
21. R. A. Helliwell, *Whistlers and Related Ionospheric Phenomena* (Stanford Univ. Press, Stanford, Calif., 1965).
22. D. B. Muldrew, *J. Geophys. Res.* **70**, 2635 (1965).
23. L. H. Brace and B. M. Reddy, *ibid.*, p. 5783.
24. D. S. Lund, W. Calvert, T. E. VanZandt, in preparation.
25. G. E. K. Lockwood, *Can. J. Phys.* **41**, 190 (1963); **43**, 291 (1965); W. Calvert and G. B. Goe, *J. Geophys. Res.* **68**, 6113 (1963).
26. W. Calvert, in *Electron Density Profiles in the Ionosphere and Exosphere*, J. Frihagen, Ed. (North-Holland, Amsterdam, 1966), p. 281; W. Calvert and T. E. VanZandt, *J. Geophys. Res.* **71**, 1799 (1966).
27. J. A. Fejer and W. Calvert, *ibid.* **69**, 5049 (1964); J. P. Dougherty and J. J. Monaghan, *Proc. Roy. Soc. London Ser. A* **289**, 214 (1965); W. D. Deering and J. A. Fejer, *Phys. Fluids* **8**, 2066 (1965); P. A. Sturrock, *ibid.* p. 88; J. Nuttall, *J. Geophys. Res.* **70**, 1119 (1965); ———, *Phys. Fluids* **8**, 281 (1965).
28. J. S. Belrose and R. E. Barrington, *Radio Sci.* **69D**, 69 (1965); D. A. Gurnett *et al.*, *J. Geophys. Res.* **70**, 1665 (1965).
29. R. E. Barrington, J. S. Belrose, G. L. Nelms, *J. Geophys. Res.* **70**, 1647 (1965).
30. R. E. Barrington, J. S. Belrose, W. E. Mather, *Nature*, in press.

Internal Clocks and Insect Diapause

Insects use photoperiodic information to bring their growth and dormant periods into harmony with season.

Perry L. Adkisson

Many insects have internal "clocks" that measure with utmost precision the duration of light and dark in each day. This photoperiodic information is used by the insects to perceive changes in season. The clock is especially important to phytophagous insects living in temperate zones, for they must adapt to seasonal change or perish. Their growth and reproductive phases must be synchronized with favorable seasons of climate and with the availability of host plants, and unfavorable seasons must be bridged by a dormant condition called diapause if the population is to survive. Furthermore, the insects must make preparations for the unfavorable season well in advance of its occurrence. Changes in the lengths of the days provide the information they need in order to make such adjustments.

Seasonal Synchronization

Since Garner and Allard (*1*), Marcovitch (*2*), and Rowan (*3*) discovered that the photoperiod plays an important role in enabling many plants and animals to synchronize their activities with the seasons, many examples of this phenomenon in insects have been reported (*4–6*). Withrow computed that organisms must have a clock that measures day length with a precision of 1 to 3 percent if they are to measure

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seasonal time to 1 week and of 4 to 12 percent for an accuracy of 1 month (*7*). The insect clock has a precision such that seasonal events, for example the onset of diapause in a given population, may occur regularly each year at almost the same date, plus or minus 2 or 3 days.

The control of diapause in the pink bollworm provides an excellent example of precision in reaction to changes in the photoperiod. The pink bollworm, the larva of the tiny moth *Pectinophora gossypiella* Saunders, is a common pest of cotton in the southwestern United States and Mexico. Typically, the female deposits her eggs on the outer surfaces of the cotton boll. After five or more days of incubation the egg hatches, and the newly emerged larva immediately burrows into the boll. For the first few days, the larva remains either in the boll carpel or along its inner surface. Later, the larva burrows deep into the boll, searching out the seed for nutriment. At the end of the larval feeding period, the insect may take either of two routes of development. During the long days of summer, the larva may eat its way out of the boll, drop to the soil, and pupate immediately; shortly there appears a new generation of moths. Alternatively, during the short days of fall, the larva may remain inside the boll and spin a silken hibernaculum. Growth and development are arrested, and the larva enters a state of diapause which persists until the following spring. Once the day length increases beyond a critical value for the species, growth and development resume, and within a few days the year's first moths appear.

The growth, development, reproduction, and diapause of the pink bollworm are confined to the appropriate seasons as a direct result of the insect's response to photoperiod. This has been clearly demonstrated by observations in the field and laboratory (*8–10*). The relationship between the photoperiod and the seasonal occurrence of the diapausing and nondiapausing stages of the pink bollworm is shown by Fig. 1. The first diapausing larvae to appear in the population each year develop from eggs deposited in the last 2 or 3 days of August. The onset of diapause in the population occurs at almost exactly the same date each year (*11*). During the first days of September, the incidence of diapause is rather low; then, with the arrival of the autumnal equinox, the percentage of diapausing larvae in the population drastically increases. Practically every larva in the population is in diapause by mid-November, and growth and development are not resumed until after the vernal equinox, when the days again exceed the critical length (*9–11*).

Growth and reproduction of the pink bollworm are confined to the seasons of year in which days, as measured from sunrise to sunset, are somewhat longer than 12.5 hours. When days become shorter than this, diapause intervenes.

Precision of Time Measurement

The photoperiodic responses of pink bollworms are sharply defined when populations are raised in the laboratory at 27°C on an artificial diet with a range of photoperiods similar to those in nature (*11*). The transition from a short- to a long-day response occurs when the number of hours of light per day increases from 13 to 13.25 (Fig. 2). Thus diapause is induced by photoperiods having 13 hours of light or less and prevented by photo-

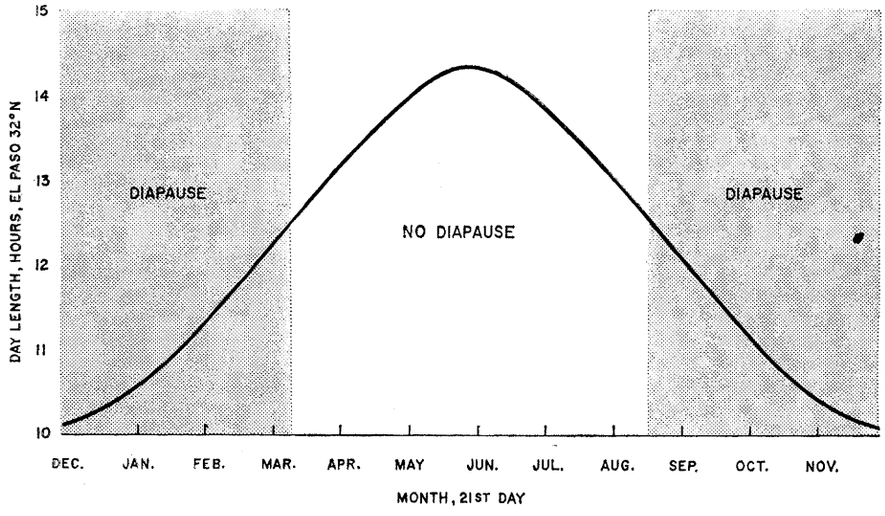

Fig. 1. Relationship of photoperiod to the seasonal occurrence of the nondiapausing (growth and reproductive) and diapausing (dormant) phases of the pink bollworm. Growth and reproduction are confined to the long days of spring and summer, and diapause is induced and stabilized by the short days of fall and winter.

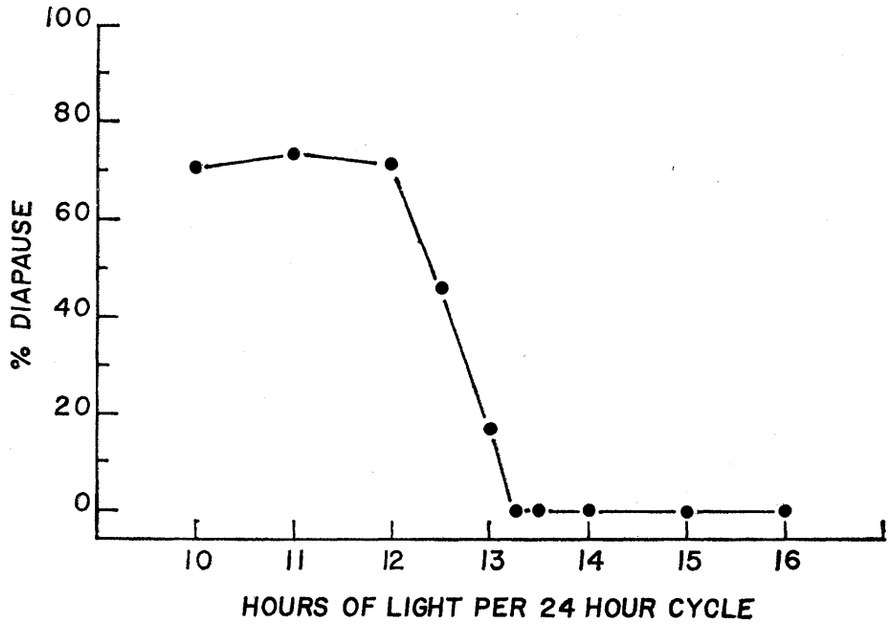

Fig. 2. Incidence of diapause in pink bollworm populations reared in selected photoperiods in 27°C. Diapause was induced in photoperiods having 13 hours or less of light per 24-hour cycle and prevented by those having 13.25 hours or more. [Adapted from *11*].

periods having 13.25 hours or more. The difference between the reactions to photoperiods whose light-times (hereafter referred to as "days") differ by only 15 minutes shows the remarkable precision with which the various responses controlling insect diapause are elicited by slight changes in the photoperiod. Control of diapause as precise as that in the pink bollworm has been recently demonstrated in other insect species (*12, 13*). The photoperiodic response of the pink bollworm appears to meet the order of precision which Withrow (*7*) computed to be demanded for measuring seasonal time within 1 week.

The response of the pink bollworm to different photoperiods is shown in Fig. 2. These data represent averages calculated from a series of tests in which 600 to over 800 pink bollworms were tested in each photoperiod. The pink-bollworm population is a heterogeneous group of individuals for which the critical or transitional photoperiod, that is, the photoperiod that induces diapause, may vary from L13 D11 to L12 D12. [For the sake of convenience, photoperiodic regimens are described in this article in terms of their daily periods of light (L) and dark (D); that is, L12 D12 describes a photoperiod consisting of 12 hours of light (or day) and 12 of dark (or night).] In this particular population, originally collected near El Paso, Texas, slightly fewer than 20 percent of the individuals diapause in response to a photoperiod of L13 D11. Others do not diapause until the daily period of light is shorter (or, conversely, that of dark is longer). For almost 30 percent of the population the transitional photoperiod is nearer L12.5 D11.5; and for the remainder of the population it is between L12.5 D11.5 and L12 D12. Nevertheless, at 27°C, nearly 30 percent of the population fail to respond to the photoperiods which usually induce diapause. For diapause to occur in all larvae, the pink bollworms must be raised in short days at temperatures of 20°C or less.

The heterogeneity of the pink bollworm population with regard to the critical photoperiod has great survival value for the population, for it insures that diapause will be spread over a period of several weeks. Since both induction and termination of diapause in the pink bollworm are under photoperiodic control, the spring emergence of the moths that have developed from the overwintering larvae is also spread over a period of several weeks. This ensures that no single, sudden catastrophic happening in the environment will kill all the members of a population.

Setting the Clock

A clock has no value unless it can be set to the correct time. The temporal cues which the insects receive in the form of the daily photoperiod serve to adjust all the individual clocks in the population to the seasonal time. The seasonal time is compared daily to the inherited timescale of the insect. In the pink bollworm, for example, this inherited time-scale—the critical photoperiod—is near L13 D11. This is the pivotal photoperiod around which the short- or long-day response revolves.

Bünning and Joerrens (*14*) suggested that the photoperiodic induction of diapause in *Pieris brassicae* (L.) is controlled by an endogenous diurnal rhythm. This rhythm consists of two half-cycles which Bünning terms "photophil," or light-liking, and "scotophil," or dark-liking. Diapause is inhibited when days are long because the daily light period extends into the scotophil part of the endogenous cycle and it is induced when the light period is restricted entirely to the photophil part of the cycle. Bünning assumes that the endogenous rhythm is entrained, or phased in nature by the light-signal of dawn. Excellent discussions of the merits of Bünning's hypothesis have been published by Pittendrigh and his co-workers (*15*).

Lees (*13*) has suggested another hypothesis, that it is the duration of the dark, and not of the light, in the photoperiod that is crucial to induction. He suggests, on the basis of results with the aphid *Megoura viciae* Buckton, that the insect clock measures only the interval between periods of light, that is, the duration of the night. It receives temporal cues from the onset of darkness, or dusk, and the onset of light, or dawn. According to Lees, the light abolishes the effects of the previous exposure to the dark provided the reactions to the dark have not been in progress for a period longer than the critical period.

That the photoperiod provides temporal cues for the insect clock has not been doubted. The question has been which components of the photoperiod —the light, the dark, or both—are important to phasing the reaction. Answers have been sought in experiments, with insects and plants, in which the nights of photoperiods having days of various durations were interrupted at certain intervals with periods of light varying in duration from a few minutes to 1 or 2 hours (*6, 11, 13–18*). I have recently completed a long series of such experiments. Each photoperiod was repeated four times with a total of 600 to 800 pink bollworms, which had been raised at 27°C on artificial diets. The nights of these selected photoperiods were interrupted at various times by 1-hour periods of light (light interruptions). Results of a preliminary analysis of the first of these experiments, which involved photoperiods with days of 6, 10, 12, and 13 hours (Figs. 3 and 4) were reported in 1964 (*11*). Results of experiments completed to the present are presented in Figs. 3, 4, and 5.

The data without exception show a bimodal configuration. Light interruptions of 1 hour given early or late in the night have different effects from those given during the middle of the night. The effect of a light interruption given during the middle of a long night that followed a short day was not simply to divide the dark into two short nights (Fig. 3), for such an interruption inhibited diapause in the L4 D20 photoperiod but induced diapause in L8 D16. Thus the action of light is dependent upon the time at which it is introduced into the daily cycle, and the times of greatest sensitivity to light in the dark period are determined by the duration of the day.

If the curves for these responses (Figs. 3 and 4) are examined only on the basis of the ability of light interruptions to inhibit diapause, a consistent pattern of response is evident. The greatest inhibition of diapause occurred when light interruptions were given either 8 to 10 hours after dusk or 8 to 10 hours before dawn. Therefore this period of sensitivity could be identified with reference to either dusk or dawn.

These results also show that the length of the day in the photoperiod is not of prime importance in determining whether diapause is induced or is inhibited. Diapause was induced or inhibited as effectively in photoperiods having 5 hours of light (L4 D20 with 1-hour light interruption) as in those having 14 hours of light per day (L13 D11 with 1-hour light interruption). The important factor, therefore, appears to be the time in the cycle at which the insect is exposed to light.

If it is not the total time of light in

the cycle that is important, then what is the action of light in the timing process? Apparently, inhibitory light interruptions presented early in the night acted as the terminator of the day, or as "dusk," and those presented late in the night acted as the initiator, or as "dawn." Thus the effect of the light interruption early in the night was to "reset" the dusk, and that of the one offered late was to "reset" the dawn.

There apparently was an interaction between the beginning or end of day and the 1-hour light interruption so that, according to the terminology of Pittendrigh and Minis (*17*), asymmetric skeleton photoperiods were formed. Good examples are the experiments involving the 12-hour day (Fig. 4; Table 1). Diapause was almost completely prevented in the regimen of L12 D3 L1 D8, in which the light interruption was given early in the night, during hour 16. This regime provided an asymmetric skeleton of L16 D8. In this case, the light interruption apparently functioned to extend the main light period. Diapause also was prevented by the regimen of L12 D10 L1 D1, in which the inhibitory light pulse was given 1 hour before the beginning of the main light period. This formed an asymmetric skeleton of L14 D10. In this case, however, the inhibitory light period apparently acted as dawn. Other examples also are provided in Table 1, which gives the light-dark regimens, plus the asymmetric skeleton photoperiods formed, which produced maximum inhibition or induction of diapause for each of the photoperiods reported in Figs. 3, 4, and 5.

The light-dark regimens having 6-, 10-, 12-, and 13-hour days were the first experiments performed. In these experiments, maximum inhibition of diapause always occurred when the inhibitory light preceded or ended an uninterrupted dark period of 8 to 10 hours' duration. These results led to the conclusions that the duration of the dark period is critical to the diapause response and that the response was largely independent of the duration of the main light period (*11*). Lees (*13*) arrived at a similar conclusion for the photoperiodic control of the sexual forms of the *Megoura* aphid. However, it now appears that this conclusion may not be entirely valid. Certain results reported in Figs. 3, 4, and 5 and summarized in Table 1 indicate that the duration of the dark is no more important than the duration of the light period.

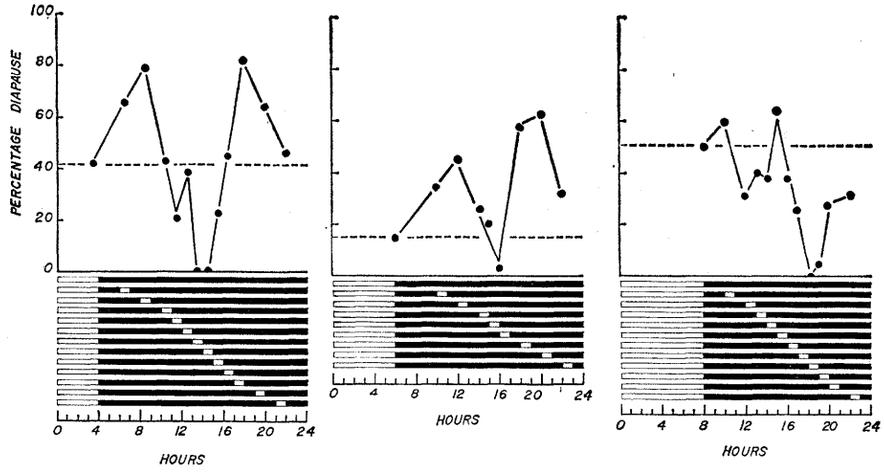

Fig. 3. Effects on diapause of 1-hour light interruptions made at various times during the nights of diapause-inducing photoperiods of L4 D20, L6 D18, and L8 D16. The horizontal dashed line in each panel serves as a reference point showing the amount of diapause produced by each of the three photoperiods when the night was not interrupted. The light interruptions may inhibit or induce diapause depending on the time in each cycle at which they are made. [Data for L6 D18 is adapted from *11*]

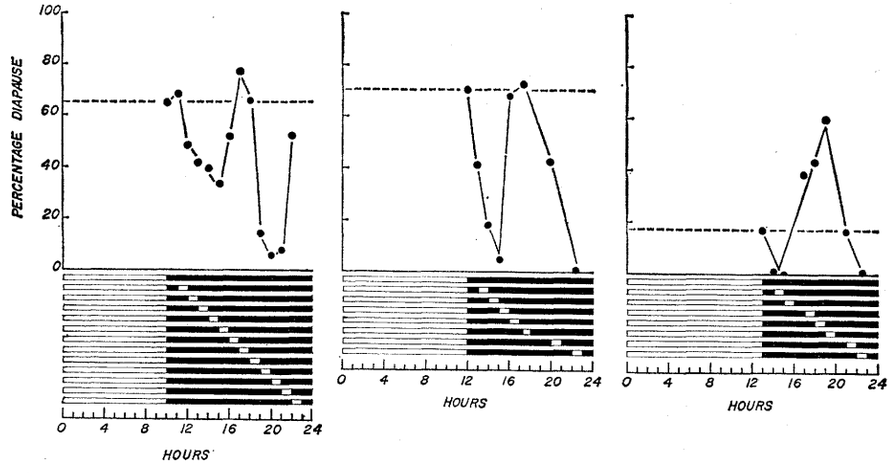

Fig. 4. Effects on diapause of 1-hour light interruptions made at various times during the nights of diapause-inducing photoperiods of L10 D14, L12 D12, and L13 D11. The horizontal dashed line in each panel serves as a reference point showing the amount of diapause produced by each of the three photoperiods when the night was not interrupted. [Adapted from *11*]

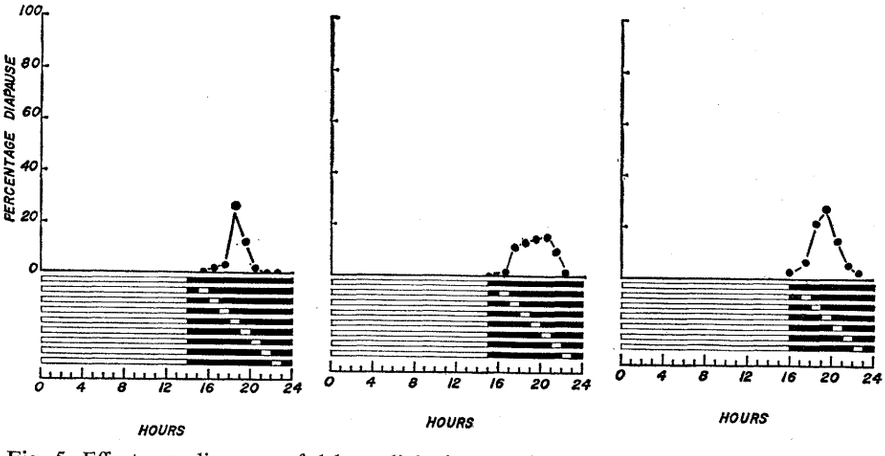

Fig. 5. Effects on diapause of 1-hour light interruptions made at various times during the nights of diapause-preventing photoperiods of L14 D10, L15 D9, and L16 D8. Certain of these interruptions caused significant percentages of the larvae to diapause.

It is evident that the action of the asymmetric skeleton photoperiods as inducers or inhibitors of diapause is intimately associated with the length of the days. Several examples are provided in Table 1. In the experiments involving the 8-hour day, maximum induction of diapause was produced by the light interruption ending at hour 16. This pulse provided an asymmetric skeleton of L16 D8 and, according to Fig. 2, should have prevented diapause. The same asymmetric skeleton, L16 D8, may also be formed by a 1-hour light interruption ending at hour 16 in regimens with days of 12, 13, and 14 hours; however, in these cases diapause was almost completely prevented.

Other examples are the asymmetric skeleton photoperiods of L17 D7 and L18 D6 (Table 1). When these skeletons were formed by light interruptions made during nights following days of 12 and 13 hours (Fig. 4) maximum induction of diapause occurred. However, when the day was extended to 14, 15, or 16 hours (Fig. 5), light interruptions which formed asymmetric skeletons of L17 D7 or L18 D6 prevented diapause. Thus it is apparent that asymmetric skeleton photoperiods having the same duration of uninterrupted darkness, that is, 6, 7, or 8 hours, under one circumstance may induce diapause, whereas under another they may produce almost complete inhibition. These responses produced by the asymmetric skeleton photoperiods were dependent on the length of the day and not on the total number of hours of light in 24 hours.

These results furnish convincing proof that it is not altogether the duration of the dark period that is crucial to the photoperiodic response of the pink bollworm. It seems more likely that within the 24-hour day the duration of the light and that of the dark are of equal importance. Apparently, the light and dark fractions of the photoperiod are intimately associated in controlling the photochemical reactions that determine whether diapause is to begin or whether the insect is to continue development. The presence of light at one time in the daily cycle may induce diapause, whereas at another it may inhibit diapause. The times during the daily cycle at which light may act as an inhibitor (or inducer) of diapause are determined by the length of the day.

The responses of the pink bollworm in this series of experiments are generally in agreement with Bünning's hypothesis (*14, 18*) that time-measurement is controlled by a photoperiodically entrained circadian rhythm. Apparently the responses are not those of a single "interval-timer" which responds only to a critical duration of the dark period. However, the present results may not preclude the possibility that there are two interval-timers involved, one which measures the day and another which measures the night. There is as yet no direct evidence of a circadian rhythm, such as that in the photoperiodic responses of certain plants and animals (*19, 20*), for the photoperiodic control of diapause.

I presented, in an earlier paper (*11*), a schematic example showing how the changes in the sensitivity of the pink bollworm to light could be used to explain the seasonal onset of diapause. This example was based on Bünning's premise (*14, 18*) that photoperiodic responses have a "photophil" and a "scotophil" phase. If light falls into the scotophil, the reaction is the opposite of what it would be if the light were restricted entirely to the photophil. This schema still appears to hold true. The pink bollworm does not diapause in photoperiods from L13.25 D10.75 to L16 D8 because the times at which light is most effective in inhibiting diapause occur during the day. Accordingly, diapause is induced by photoperiods from L10 D14 to L13 D11 because the times in the daily cycle during which light may inhibit diapause occur during the night. The insect normally is not exposed to light during this time, so diapause ensues. This conclusion is implicit in the results presented in Figs. 3, 4, and 5 and still appears to be the most valid explanation of the seasonal occurrence of diapause in the pink bollworm population.

Table 1. The light-dark regimens which produced the maximum and minimum incidences of diapause in the light-interruption experiments. The asymmetric skeleton photoperiods have been derived on the assumption that there is an interaction between the day and the 1-hour light interruptions so that a light interruption given early in the night is taken by the insect as being the end of day, whereas one given late in the night is taken as the beginning of the day. The portion of each skeleton which functioned as the day is shown by the underline. For detailed discussions of the theoretical aspects of this assumption see Pittendrigh *et al.* (*15, 17*).

Day (hr)	Maximum inhibition of diapause			Maximum induction of diapause		
	Light-dark regimen	Asymmetric photoperiod formed	Diapause (%)	Light-dark regimen	Asymmetric photoperiod formed	Diapause (%)
4	L4 D9 L1 D10	L14 D10	0.0	L4 D4 L1 D15	L9 D15	79.1
	L4 D10 L1 D9	L14 D10	.0	L4 D13 L1 D6	L11 D13	83.0
6	L6 D10 L1 D7	L14 D10	3.2	L6 D6 L1 D11	L13 D11	45.5
				L6 D14 L1 D3	L10 D14	61.9
8	L8 D4 L1 D11	L13 D11	30.6	L8 D2 L1 D13	L11 D13	59.8
	L8 D10 L1 D5	L14 D10	0.0	L8 D7 L1 D8	L16 D8	63.7
10	L10 D5 L1 D8	L16 D8	32.8	L10 D1 L1 D12	L12 D12	69.1
	L10 D10 L1 D3	L14 D10	6.7	L10 D7 L1 D6	L17 D7	77.1
12	L12 D3 L1 D8	L16 D8	4.4	L12 D5 L1 D6	L18 D6	72.2
	L12 D10 L1 D1	L14 D10	0.3			
13	L13 D2 L1 D8	L16 D8	0.7	L13 D6 L1 D4	L18 D6	58.6
	L13 D9 L1 D1	L15 D9	.1			
14	L14 D1 L1 D8	L16 D8	0.0	L14 D4 L1 D5	L19 D5	27.3
	L14 D7 L1 D2	L17 D7	.0			
15	L15 D1 L1 D7	L17 D7	1.6	L15 D5 L1 D3	L19 D5	15.2
	L15 D7 L1 D1	L17 D7	1.1			
16	L16 D1 L1 D6	L18 D6	7.7	L16 D3 L1 D4	L20 D4	27.8
	L16 D6 L1 D1	L18 D6	3.2			

Rate of Induction of Diapause

Lees (*5, 21*) has suggested that some substance capable either of preventing or of inducing diapause is synthesized during one fraction of the photoperiod and is destroyed or otherwise inactivated during the complementary fraction, and that synthesis and removal must be a cumulative process, with some residue of the active substance being carried over from one cycle to the next. Lees concluded that although some mechanism must exist for accumulating such residues from successive photoperiods, the nature of the process in insects is not understood.

Effects of short-day exposures in inducing diapause in the pink bollworm may be reversed by exposing the larvae to long days until they are well into the last instar (*22*). Similarly, effects of long-day exposures may be nullified by subsequent short-day treatments. This indicates that if there is some active substance being formed under one set of photoperiodic conditions, its activity may be reversed or nullified by subsequent exposure to opposing photoperiods.

We have obtained results within the past year which apparently are in agreement with Lees's hypothesis (*5, 21*) that there must be some mechanism in the pink bollworm for accumulating the products of successive photoperiods (*23*). For example, diapause may be induced in as many as 30 percent of the larvae by as few as four inductive photoperiods given in the first days of larval life (Fig. 6). This is true, however, only if the eggs from which the larvae develop are hatched in continuous light (LL) and if the larvae are placed back in continuous illumination immediately following the four inductive treatments. By this technique, only six or seven L12 D12 photoperiods given during the first week of the larval period will produce as high an incidence of diapause as when this photoperiod is given during the entire larval feeding period of approximately 15 to 20 days. However, if the larvae, instead of being returned to continuous light, are subjected to photoperiods of L14 D10 or L16 D8 a considerably greater number of inductive photoperiods must be given to produce comparable percentages of diapausing larvae in the test populations. This finding indicates that six or seven photoperiods of L12 D12 are not sufficient to induce diapause irreversibly. However, this number apparently is sufficient to synchronize the inductive processes involved in diapause, and this synchrony is maintained by the larvae in the aperiodic conditions of constant illumination. Therefore, the larvae continue to respond as if they were still in the original synchronizing photoperiod, and the incidence of diapause in this population will be as great as in one kept in a photoperiod of L12 D12 until the end of the larval developmental period.

Table 2. Number of photoperiods of L12D12 required to induce diapause in pink bollworm larvae. After exposure to L12 D12, insects were transferred to continuous light in which they were kept until the larvae were 40 days old.

Photoperiods of L12 D12 (No.)			Insects undergoing diapause (%)
Eggs	Larvae	Total	
1	0	1	26.1
2	0	2	22.5
3	0	3	18.9
4	0	4	36.3
5	0	5	44.6
5	1	6	62.5
5	2	7	79.4
5	3	8	69.1
5	5	10	65.9
5	6	11	87.8
5	20	25	78.7

The processes that induce diapause may be set into motion in the egg. Exposure of eggs to L12 D12 for the duration of the incubation period produced diapause in nearly 50 percent of the resulting larval population (Table 2). When the inductive treatment was extended to include the larvae until they were 2 days old, the maximum number of insects were induced to enter diapause. In all these experiments the insects were placed in continuous light after the inductive treatments. My co-workers and I have recently determined that their response is approximately the same as that of insects placed in continuous darkness after treatment. These results show that as few as seven inductive photoperiods given to eggs and first-instar larvae will result in diapause in as many of the pink bollworms as will continuous exposure to the photoperiod to the end of the last larval instar; however, this is true only if after treatment the young larvae are placed in an aperiodic environment in which there are no temporal cues. Again, this suggests that once the endocrine mechanism involved in the photoperiodic response is synchronized with a certain light-dark signal for a period of a few days, the pattern established is maintained in the absence of temporal cues from the environment. Moreover, if the insect is transferred to a different photoperiod, the endocrine mechanism soon becomes adjusted to the new signal. The pattern of growth and development thereafter may be changed in accordance with the new directions provided by this photoperiod.

This response might also be interpreted as implicating a photoperiodically entrained circadian rhythm in the diapause of the pink bollworm. This rhythm might be entrained by as few as six or seven successive photoperiods experienced during the egg and the early larval stages. The rhythm would remain in phase with the entraining photoperiod even if the insect was later moved to an aperiodic environment in which it received no temporal cues. This "time-memory" would be very beneficial to the pink bollworm, since diapause could be controlled by the photoperiods experienced by the insect while in the egg or early larval stage. Both stages are found in situations in which the intensity of the natural light is well above the minimum required for the response. This sensitivity in early stages would minimize the hazard that would result if the intensity of light received by older larvae, feeding deep inside the cotton boll, should fall below the threshold level.

Is a Circadian Rhythm Involved?

Results of experiments in which the night has been interrupted with brief light periods have been interpreted as furnishing evidence for a circadian rhythm in the photoperiodic control of insect diapause (*14, 15, 17, 18*). The results presented in the preceding section also might implicate a circadian rhythm since they imply that the pink bollworm has a "time-memory" that allows previous photoperiodic experiences to be carried over in aperiodic environments.

To pursue this question further, experiments were conducted along the lines suggested by K. C. Hamner *et al.* for plants (*19*) and by W. M. Hamner for birds (*20*). The experimental procedure incorporates short light or dark periods into photocycles ranging from 12 to 72 hours in duration. Moths were exposed to L8 D16 and L14 D10. Eggs deposited by these moths were incubated (5 days) in the same photoperiod as that in which the parents had been kept, and after hatching the larvae were kept in the same regimen for

4 days before being transferred to longer cycles. This number of successive light-dark regimens is sufficient to entrain the larvae to the selected photoperiod, either L8 D16 or L14 D10. The short light (or dark) periods of the 48- and 72-hour cycles were arranged so that they always appeared in phase with the appropriate component of the original entraining 24-hour photoperiod. In the 12-, 36-, and 72-hour cycles, the short light (or dark) component appeared alternately in phase and out of phase by one-half of the period. If there is a circadian component in the diapause of the pink bollworm, the responses produced by the 48- and 72-hour cycles should be approximately the same as those produced by the 24-hour cycle. Conversely, the response produced by the 12-, 36-, and 60-hour cycles should be entirely different; that is, if diapause is induced by the 24-hour light-dark regimen, it should also be induced by the 48- and 72-hour cycles but inhibited by those of 36 and 60 hours. The plot of the data should then show a rhythmical configuration.

In our experiments, however, diapause was inhibited in all cycles that varied from 24 hours. There was no evidence for a circadian rhythm in the photoperiodic control of diapause in insects kept in photocycles having 8-hour light (Fig. 7) or 16-hour dark periods (Fig. 8). Similar results were obtained with the 10-hour dark period, in that there was no indication of rhythmical response. In this experiment diapause was completely prevented regardless of the duration of the cycle.

The responses of the insects in the 48- and 72-hour cycles (Figs. 7 and 8) were also strikingly different from those of larvae transferred from an entraining photoperiod to continuous light (Table 2 and Fig. 6). In the latter instance, seven successive entraining photoperiods were sufficient to induce diapause in the maximum number of in-

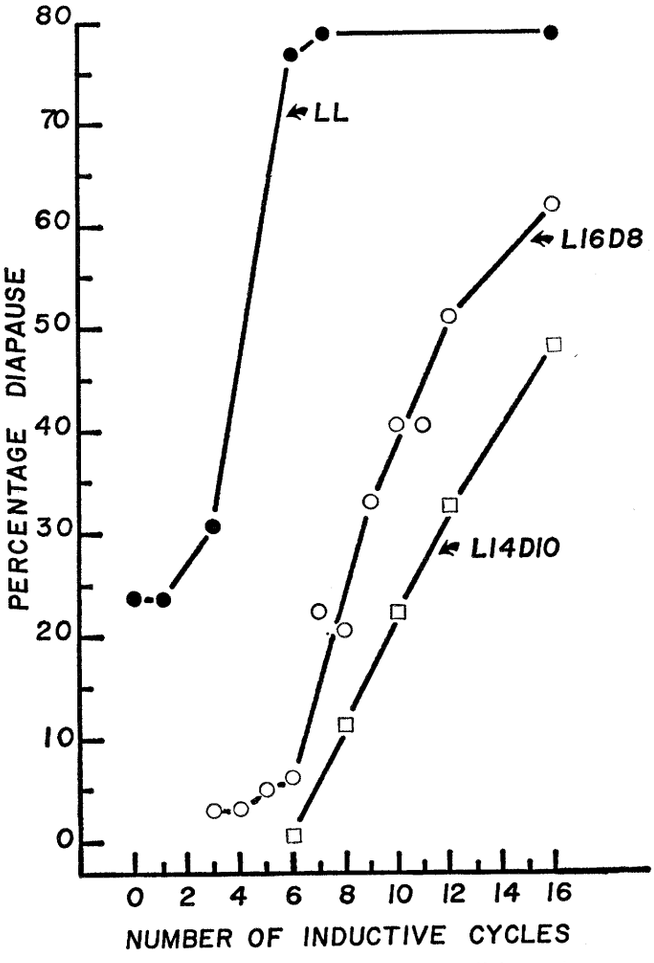

Fig. 6 (above). Effects of the post-treatment light regimen on the incidence of diapause in pink bollworm larval populations which had been subjected to various numbers of inductive L12 D12 photoperiods.

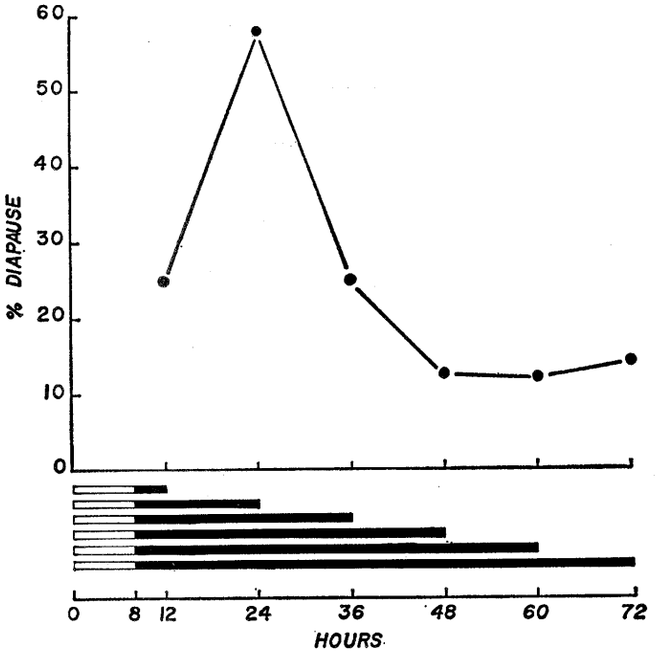

Fig. 7 (top right). Incidence of diapause in pink bollworm populations subjected to nine successive photoperiods of L8 D16 before being transferred to cycles of 12, 24, 36, 48, 60, and 72 hours in duration. The duration of the days in each cycle was always 8 hours.

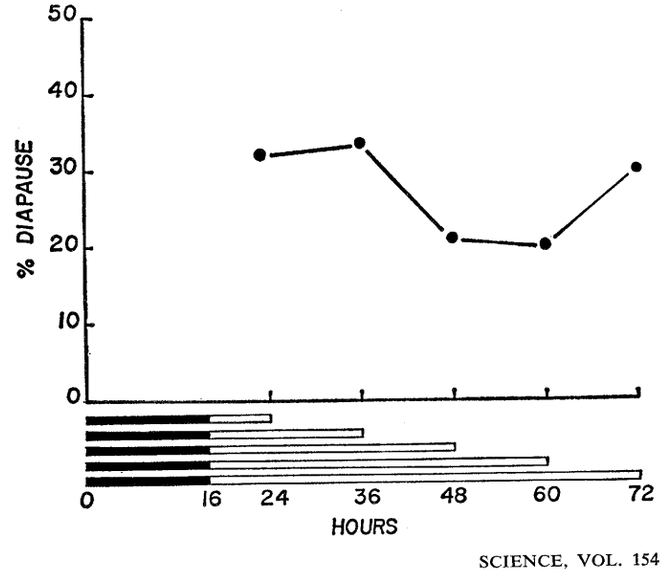

Fig. 8 (bottom right). Incidence of diapause in pink bollworm populations subjected to nine successive photoperiods of L8 D16 before being transferred to cycles of 24, 36, 48, 60, and 72 hours. The duration of the nights in each cycle was always 16 hours.

sects. However, when larvae were transferred from an inductive photoperiod of L8 D16 into 48- and 72-hour cycles, the prior inductive experience was "damped-out" (Fig. 7) even though a temporal cue appeared in exact phase with that of the original entraining photoperiod every 2 days in the 48-hour cycle and every 3 days in the 72-hour cycle. If there were a circadian rhythm involved in the photoperiodic control of diapause in the pink bollworm, it should have been evident in these results. Thus, although there is considerable circumstantial evidence that a circadian rhythm is involved in the photoperiodic control of insect diapause, direct experimental proof of its existence is still lacking.

Apparently, one of the functions of the insect clock is to program the neurosecretory activity of the brain. First evidence of this was provided by Lees (*24*), who controlled the sexual forms of the aphid *Megoura* by illuminating the mid-area of the brain. Williams and I (*12*) subsequently showed that the action of the photoperiod in controlling the pupal diapause of the oak silkworm, *Antheraea pernyi* Guer., is directly on the insect brain (*12*). When the brain of the oak silkworm pupa was surgically transplanted to the "tail-end" of the pupa, photosensitivity was transferred from the cephalic to the posterior region.

Thus, in photoperiods appropriate for growth and development, it appears that the brain is activated and the brain hormone is released. This hormone stimulates the prothoracic glands. These glands in turn secrete a second hormone, ecdysone, which acts on the cells to bring about growth and development. If photoperiods are inappropriate, no brain hormone is released; growth and development are therefore arrested, and diapause ensues (*12*).

Many insects have some physiological mechanism, or clock, which responds with utmost precision to changes in the duration of light and dark in each day. This photoperiodic information is used to ensure that the growth, reproductive, and dormant phases of the insect occur in the appropriate seasons. In the case of the pink bollworm, growth and reproduction are confined to the long days of spring and summer, and the winter season is bridged by a dormant period called diapause, which occurs in response to the short days of fall.

The insect clock receives its temporal cues from the "lights-on" of dawn and the "lights-off" of dusk. The photoperiodic control of diapause appears to depend not on the absolute duration of either the light or the dark component of the photoperiod, but on their relative duration within the 24-hour cycle. Apparently, the light and dark components of the photoperiod are intimately associated in controlling the photochemical reactions involved in the diapause process. The presence of light at one time in the daily cycle may induce diapause, whereas at another time it may inhibit diapause. Whether light interruptions made during the night act as inhibitors or inducers of diapause is dependent on the length of the day of the photoperiod.

Although there is considerable circumstantial evidence that implicates a photoperiodically entrainable circadian rhythm in insect diapause, direct experimental proof is lacking.

The clock is apparently located in the insect brain, where it programs the flow of brain hormone necessary for the stimulation of growth and development.

References and Notes

1. W. W. Garner and H. A. Allard, *J. Agr. Res.* **18**, 553 (1920).
2. S. Marcovitch, *ibid.* **27**, 513 (1924).
3. W. Rowan, *Proc. Boston Soc. Nat. Hist.* **38**, 147 (1926).
4. H. G. Andrewartha, *Biol. Rev.* **27**, 50 (1952); J. de Wilde, *Ann. Rev. Entomol.* **7**, 1 (1962); A. S. Danilevskii, *Photoperiodism and Seasonal Development of Insects*, J. Johnston, Transl. (Oliver and Boyd, London, 1965).
5. A. D. Lees, *Ann. Appl. Biol.* **40**, 487 (1953); *The Physiology of Diapause in Arthopods* (Cambridge Univ. Press, Cambridge, 1955); in *Photoperiodism and Related Phenomena in Plants and Animals*, R. B. Withrow, Ed. (AAAS, Washington, D.C., 1959), p. 585.
6. S. D. Beck, *Biol. Bull.* **122**, 1 (1962); *Bull. Entomol. Soc. Amer.* **9**, 8 (1963).
7. R. B. Withrow, in *Photoperiodism and Related Phenomena in Plants and Animals*, R. B. Withrow, Ed. (AAAS, Washington, D.C., 1959), p. 439.
8. M. J. Lukefahr, L. W. Noble, D. F. Martin, *U.S. Dept. Agr. Tech. Bull.* No. 1304 (1964).
9. P. L. Adkisson, *J. Econ. Entomol.* **54**, 1107 (1961); ———, R. A. Bell, S. G. Wellso, *J. Insect. Physiol.* **9**, 299 (1963); S. G. Wellso and P. L. Adkisson, *Ann. Entomol. Soc. Amer.* **57**, 171 (1964).
10. P. L. Adkisson, D. L. Bull, W. E. Allison, *J. Econ. Entomol.* **53**, 791 (1960); D. L. Bull and P. L. Adkisson, *ibid.*, p. 793.
11. P. L. Adkisson, *Amer. Naturalist* **98**, 357 (1964).
12. C. M. Williams and P. L. Adkisson, *Biol. Bull.* **127**, 511 (1964).
13. A. D. Lees, in *Circadian Clocks*, J. Aschoff, Ed. (North-Holland, Amsterdam, 1965), p. 351.
14. E. Bünning, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 249 (1960); ——— and G. Joerrens, *Z. Naturforsch.* **15b**, 205 (1960); E. Bünning, *Physiological Clocks* (Academic Press, New York, 1964).
15. C. S. Pittendrigh, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 159 (1960); ———, *Harvey Lectures Ser. 56*, 93 (1961); ——— and V. G. Bruce, in *Photoperiodism and Related Phenomena in Plants and Animals*, R. B. Withrow, Ed. (AAAS, Washington, D.C., 1959), p. 475; C. S. Pittendrigh, in *Circadian Clocks*, J. Aschoff, Ed. (North-Holland, Amsterdam, 1965), p. 276; D. H. Minis, *ibid.*, p. 333.
16. R. J. Barker, *Experientia* **19**, 185 (1963); ———, C. F. Cohen, A. Mayer, *Science* **145**, 1195 (1964); R. J. Barker and C. F. Cohen, *Entomol. Exp. Appl.* **8**, 27 (1965); A. Kleinhoote, *Arch. Neerl. Sci. Exactes Nat Ser. IIIb* **5**, 1 (1929); D. S. Farner, in *Circadian Clocks*, J. Aschoff, Ed. (North-Holland, Amsterdam, 1965), p. 357.
17. C. S. Pittendrigh and D. H. Minis, *Amer. Naturalist* **98**, 261 (1964).
18. E. Bünning, in *Photoperiodism and Related Phenomena in Plants and Animals*, R. B. Withrow, Ed. (AAAS, Washington, D.C., 1959), pp. 507, 531.
19. L. T. Blaney and K. C. Hamner, *Botan. Gaz.* **119**, 10 (1957); K. C. Hamner, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 269 (1960); ——— and A. Takimoto, *Amer. Naturalist* **98**, 295 (1964).
20. W. M. Hamner, *Science* **142**, 1294 (1963); *Nature* **203**, 1400 (1964); in *Circadian Clocks*, J. Aschoff, Ed. (North-Holland, Amsterdam, 1965), p. 379.
21. A. D. Lees, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 261 (1960).
22. R. A. Bell and P. L. Adkisson, *Science* **144**, 1149 (1964).
23. The data reported in Table 1 and Fig. 6 are from previously unpublished experiments performed in my laboratory by R. A. Bell and E. C. Berry.
24. A. D. Lees, *J. Exp. Biol.* **41**, 119 (1964).

25. Research reported from my laboratory was conducted in cooperation with the Entomology Research Division, USDA. Supported in part by funds from NSF grant GB-1493.